REMARKS

Entry of the foregoing amendments for the above-identified application is respectfully requested. The instant amendment is believed to be consistent with 37 C.F.R. §1.116 since the amendments place the claims in condition for allowance. At the very least, they simplify the issues on appeal. The amendments are made to overcome the §112(1) and (2) rejections. No new issues are presented nor would further consideration and/or search be required since dependent claims have been incorporated into the independent claims, and claim 1 has simply been divided into two separate claims.

Claims 2-5, 12 and 15 have been deleted without prejudice or disclaimer. Claim 1 has been amended to delete the recitations regarding the "fusion protein." New claims 29-49 have been added to recite the embodiments previously recited in claim 1 and claims dependent therefrom, wherein the peptide of interest is part of a "fusion protein." No new issues are raised by these new claims since the embodiments were previously within the scope of claim 1 and claims dependent therefrom. Claim 1 has also been amended to recite that the helper peptide has 5-50 amino acid residues and that it is designed to provide an isoelectric point of the peptide of interest with the helper peptide added thereto of between 8 and 12. Support for this amendment may be found at the very least in original claims 2 and 12.

The specification has been objected to as not complying with the Sequence rules. The specification has been amended to identify the sequence number for "RHHGP." Please note that, since underlining appeared in the original specification, double

underlining is used to identify the material being added. Withdrawal of this objection is respectfully requested and believed to be in order.

Claims 1-23 and 25-28 have been rejected under 35 U.S.C. §112, first paragraph, as the specification allegedly fails to enable the claimed process. This rejection is respectfully traversed.

The Official Action acknowledges that the specification enables a process of making GLP-1 and derivatives thereof. However, the specification is said not to enable a process of making any peptide. For example, it allegedly would be unpredictable which helper should be used for each peptide of interest. Excessive trial and error experimentation would allegedly be required to identify the necessary helper sequence that would provide the properties allowing the production of a highly purified peptide of interest. It is further asserted that the specification does not provide guidance for predicting what structure of helper would be suitable for a given peptide of interest. A purification scheme for a peptide with known characteristics would also allegedly require trial and error experimentation.

Applicants respectfully submit that the scope of the claims as written are fully enabled by the specification. As amended, the claims now require a helper peptide (1) having 5 to 50 amino acid residues and (2) that provides an isoelectric point of between 8 and 12 for the combined peptide of interest and helper peptide. The scope of the helper peptide has thus been narrowed to more clearly define the helper peptides useful in the instant invention.

One skilled in the art would recognize that there are various peptides having different pH values, i.e., different isoelectric points. However, a pH value possessed by a peptide to be produced may not be suitable for production by a gene recombination method. Problems of solubility and gelling, for example, may result in the recombinant method. The problems are the result of various physicochemical properties of the peptide of interest. *See, e.g.,* page 2, lines 10-19. As discussed in the specification at page 4, lines 16-20, "optimal pH of enzymatic amidation reactions is approximately from weak acid to neutral." Therefore, if a peptide of interest has a neutral to weak acid pH value, the solubility of the peptide of interest during its production is very low. The low solubility inhibits the productivity of the peptide of interest. Applicants discovered the use of "helper peptides" to be added to the peptide of interest to overcome this problem in the art. The helper peptides as claimed have 5-50 amino acid residues, and are designed so that the combined peptide of interest and helper peptide has an isoelectric point between 8 and 12. As discussed on page 10, lines 18-23:

> The helper peptide of the present invention may be prepared as appropriate depending on the physicochemical properties of the peptide of interest. For example, when the isoelectric point of the peptide of interest is neutral to weak acid and the optimum pH during the production process is also neutral to weak acid and thereby the solubility of the peptide of interest under such pH is too low, then the helper peptide is preferably designed so that the isoelectric point (pI) of the peptide of interest that has a helper peptide added thereto is 8 to 12 and more preferably 9 to 11. The helper peptide of the present invention may be added to either the N-terminal or C-terminal of the peptide of interest. The dimension (length) of a helper peptide is preferably 5 to 50 amino acid residues, and more preferably from 5 to not greater than 30 amino acid residues, but it contains at least one basic amino acid or acidic amino acid.

By changing the isoelectric point, the peptide of interest to which the helper peptide is added, is soluble during its production process. In this manner, the low solubility does not inhibit the productivity of the peptide of interest.

One skilled in the art could readily select helper peptides having 5-50 amino acid residues, which provides a pI of between 8-12 for the peptide of interest having the helper peptide added thereto. One skilled in the art can readily evaluate pI values of the helper peptide and the combined helper peptide-peptide of interest. It is well known in the art that an isoelectric point of a peptide or protein can be calculated on the basis of the amino acid composition of the peptide or protein. Thus, once the peptide to be produced is identified, and its amino acid composition is known, the isoelectric point of the peptide would be known. One skilled in the art could then readily select a helper peptide which could be used to provide an isoelectric point for the combined peptide between 8 and 12.

Therefore, no undue experimentation would be required to select an appropriate helper peptide to be used in the process of the invention.

The particular structure, function and characteristics of the peptide of interest and structure of the helper peptide are not critical to the claimed process and need not be specified in the claims. The peptide of interest is expressed as an intermediate peptide comprising the peptide of interest and a helper peptide which is added to the peptide of interest. In this intermediate peptide (or precursor peptide) the helper peptide changes the physicochemical properties, i.e., isoelectric point, of the peptide of interest so that the intermediate (or precursor) peptide can be easily, and stably purified. After purification of the intermediate peptide, the intermediate peptide is cleaved to liberate the peptide of

interest. This method does not depend upon the particular structure, function or characteristics of a particular peptide of interest or the structure of the helper peptide, but instead can be generally used by those skilled in the art for any peptide or interest. This would be clear to a person skilled in the art.

In addition to GLP-1, the specification identifies additional peptides of interest for which the claimed process may be used. *See, for example,* page 10, line 34 - page 11, lines 1 to 27. One skilled in the art could readily determine what helper peptides could be used to provide the isoelectric point of between 8-12 for the peptide of interest.

A specification may be enabled even if experimentation is necessary so long as the experimentation is not undue. Since one skilled in the art could readily select an appropriate helper peptide and determine the isoelectric points, no undue experimentation would be required to practice the invention as claimed.

The application as claimed is thus enabled by the specification. Withdrawal of this rejection of record is respectfully requested. Such action is believed to be in order.

Claims 1-23 and 25-28 have been rejected under 35 U.S.C. §112, second paragraph. This rejection is believed to have been overcome by the instant amendment.

Claim 1 is said to be confusing because it is allegedly directed to two distinct processes of making two distinct products. The first product is said to be the peptide of interest with the helper peptide added thereto, and the second, a fusion protein that has a protective peptide further added to the peptide of interest-helper peptide product. Claim 1 has been amended to be directed to a first product only, i.e., the peptide of interest with the helper peptide added thereto. New claims 29-49 have been added to be directed to the

embodiment of the second product, i.e., a fusion protein having a protective peptide added to the peptide of interest-helper peptide product.

Claim 23 is allegedly unclear because claim 1 refers to the purification of "the peptide of interest," and not "the final purified product." The claim has been amended to overcome this rejection and recite "the peptide of interest."

In view of the above, withdrawal of the rejection of claims 1-23 and 25-28 under §112(2) is respectfully requested and believed to be in order.

Claims 1-13, 18-23 and 25-27 have been rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Tarnowski et al. This rejection is respectfully traversed.

As now claimed, the claimed process requires a helper peptide having from 5 to 50 amino acid residues. The molecular weight of a peptide having 50 amino acid residues is about 6 kD. By contrast, the carrier protein of Tarnowski et al has a molecular weight of about 10 to 50 kD. Nor does Tarnowski et al disclose or suggest a helper peptide which will provide an isoelectric point of the combined peptide of interest and helper peptide of between 8-12. Tarnowski et al uses a carrier protein to easily remove impurities. This would neither disclose nor suggest the use of a helper peptide which will provide a specified isoelectric point for the combined peptide product. This reference thus fails to disclose or even suggest the claimed process for producing a peptide having a desired biological activity.

Withdrawal of the rejection of record is respectfully requested and believed to be in order.

Claims 1-13, 18-23 and 25-27 have been rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Yabuta et al. This rejection is respectfully traversed.

Yabuta et al refers to a fusion protein having an isoelectric point of between 4.9 and 6.9. This isoelectric point range is different from the claimed range of between 8-12. Nor does Yabuta et al suggest using a helper peptide to adjust the isoelectric point of the peptide of interest. Yabuta in no way suggests such a use of a helper peptide to enhance the solubility of the peptide of interest in order to enhance the productivity of the peptide of interest.

Yabuta et al thus fails to disclose or even suggest the instantly claimed process.

Withdrawal of this rejection is respectfully requested and believed to be in order.

Claims 14-17 have been rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Tarnowski et al in view of Bell et al. Claims 14-17 have been rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Yabuta et al in view of Bell et al. These rejections are respectfully traversed.

As set forth *supra*, Tarnowski et al fails to disclose or even suggest a helper peptide of the claimed size of 5 to 50 amino acid residues. Tarnowski et al and Yabuta et al both fail to disclose or even suggest using a helper peptide to provide an isoelectric point of between 8-12 for the combined peptide of interest-helper peptide. Bell does not overcome or remedy these deficiencies of Tarnowski et al or Yabuta et al. Bell is cited as teaching GLP-1 and its physiological importance. This teaching suggests nothing of the problem of producing such a protein recombinantly, e.g., solubility problems, and how to overcome it, e.g., by using a helper peptide to alter the isoelectric point of the peptide of interest.

The combinations of references thus fail to disclose or even suggest the instantly claimed processes. Withdrawal of these rejections is thus respectfully requested and believed to be in order.

Claim 28 has been rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Tarnowski et al in view of Mojsov et al. Claim 28 has also been rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Yabuta et al in view of Mojsov et al. These rejections are respectfully traversed.

As set forth *supra*, Tarnowski et al fails to disclose or even suggest a helper peptide of the claimed size of 5 to 50 amino acid residues. Tarnowski et al and Yabuta et al both fail to disclose or even suggest using a helper peptide to provide an isoelectric point of between 8-12 for the combined peptide of interest-helper peptide. Mojsov et al does not overcome or remedy these deficiencies of Tarnowski et al or Yabuta et al. Mojsov et al is cited as teaching a natural derivative of GLP-1(7-37), GLP-1(7-36)NH_2 and its physiological importance. This teaching suggests nothing of the problem of producing such a protein recombinantly, e.g., solubility problems, and how to overcome it, e.g., by using a helper peptide to alter the isoelectric point of the peptide of interest.

The combinations of references thus fail to disclose or even suggest the instantly claimed processes. Withdrawal of these rejections is thus respectfully requested and believed to be in order.

Withdrawal of the rejections of the claims under §103(a) is respectfully requested and believed to be in order.

Further and favorable action in the form of Notice of Allowance is respectfully requested. Such action is believed to be in order.

In the event that there are any questions relating to this amendment or the application in general, it would be appreciated if the Examiner would contact the undersigned attorney be telephone at 508-339-3684 so that prosecution would be expedited.

Respectfully submitted,

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Attachment to Reply and Amendment dated December 26, 2002

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Page 8, Paragraph Beginning at Line 5

Figure 15 is a drawing that shows an analytical pattern at each purification step in the cleavage process of GLP-1 (7-37) from RHHGP[G] [SEQUENCE ID NO:25] by Kex2 protease. In the figure A indicates a reverse=phase pool before cleavage with Kex2 protease, B after cleavage with the Kex2 protease, and C after PorosR2, and 1 indicates RHHGP[G] [SEQUENCE ID NO:25] and 2 indicates GLP-1 (7-37).

Page 8, Paragraph Beginning at Line 12

Figure 16 is a drawing that shows the pH dependency of the amidation reaction of RHHGP[G] [SEQUENCE ID NO:25].

Page 8, Paragraph Beginning at Line 14

Figure 17 is a drawing that shows a time course of the conversion of RHHGP[G] [SEQUENCE ID NO:25] to RHHGP-1 [SEQUENCE ID NO:25] in an amidation reaction as measured by an ion exchange HPLC, wherein 1 indicates RHHGP[G] [SEQUENCE ID NO:25] and 2 indicates RHHGP-1 [SEQUENCE ID NO:25]. Absorbance was measured at 280 nm.

Attachment to Reply and Amendment dated December 26, 2002

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Page 23, Paragraph Beginning at Line 15

RHHGP[G] [SEQUENCE ID NO:25] obtained as mentioned above was subjected to an amidation modification reaction using an amidation enzyme (B. B. R. C., Vol. 150, pp. 1275-1281, 1988, EP299790A, and the like). Under the reaction condition in Examples described below, no aggregation or gelling of RHHGP[G] [SEQUENCE ID NO:25] as the enzyme substrate and the reaction product, amidated GLP-1 (7-36)NH_2 (referred to hereinafter as RHHGP-1) [SEQUENCE ID NO:25] having a helper peptide added thereto occurs, and RHHGP-1 [SEQUENCE ID NO:25] could be produced at a high reaction rate of 98% (a recovery of 95%). These results demonstrated the usefulness of the helper peptide in the amidation enzymatic reaction conducted with a substrate of RHHGP[G] [SEQUENCE ID NO:25].

Page 24, Paragraph Beginning at Line 12

One object of the present invention on the production of GLP-1 (7-36)NH_2 is to relieve aggregation and increase solubility at the time of reaction of modification enzymes such as an amidation enzyme described above through a peptide comprising GLP-1(7-37) having a helper peptide added thereto. In order to further investigate the usefulness of the present invention, RHHGP[G] [SEQUENCE ID NO:25], RHHGP-1 [SEQUENCE ID NO:25], GLP-1 (7-37), and GLP-1(7-36)NH_2 were purified and the pH dependency of the solubility of each peptide was examined. As a result, GLP-1(7-37) was shown to have a

Attachment to Reply and Amendment dated December 26, 2002

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low solubility in the range of pH 5.0 to pH 7.0 as expected. On the other hand, RHHGP[G] [SEQUENCE ID NO:25] had a high solubility in the range of pH 5.0 to pH 7.0 as expected. On the other hand, RHHGP[G] [SEQUENCE ID NO:25] had a high solubility in the range of from pH 4.0 to about pH 6.0. The result confirmed that the substitution of RHHGP[G] [SEQUENCE ID NO:25] having a helper peptide for GLP-1(7-37) as an enzyme substrate is useful since the amidation enzyme reaction is carried out in the weak acid region.

Page 24, Paragraph Beginning at Line 30

In the experiment that investigated the pH dependency of solubility of each peptide, RHHGP[G] [SEQUENCE ID NO:25] and RHHGP-1 [SEQUENCE ID NO:25] have shown a sudden reduction in solubility at about pH 6.0 and about pH 6.4, respectively. GLP-1(7-36)NH_2 formed precipitates or microcrystals with time. Thus, it is estimated that substances capable of increasing the solubility of RHHGP[G] [SEQUENCE ID NO:25] and RHHGP-1 [SEQUENCE ID NO:25] in the neutral to weak alkali region and substances capable of increasing the solubility of the peptide of interest in the weak acid to weak alkali region would be very useful in the production process.

Attachment to Reply and Amendment dated December 26, 2002

Marked-up Copy

Page 30, Paragraph Beginning at Line 27

The obtained suspension of the inclusion body was diluted to give an OD660 value of 1000. From the liquid a 1000 ml portion was taken, to which 250 ml of 1M Tris-HCl for which pH has not been adjusted, 10 ml of 0.5 M EDTA (pH 8.0), and 1200 g of powdered urea was added, and then deionized water was added thereto to give a final volume of 5000 ml. Then HCL was used to adjust pH at 7.5 and the liquid was heated to 37° C for 2 hours. This procedure initialed the action of E. coli OmpT protease present in the inclusion body, and GP97ompPR was cleaved and RHHGP[G] [SEQUENCE ID NO:25] was released. Figure 10 is the result of the RHHGP[G] [SEQUENCE ID NO:25] that was excised from GP97ompPR and then was analyzed with reverse phase HPLC. The analysis used a YMC PROTEIN-PR column, a 10% acetonitrile solution containing 0.1% trifluoroacetic acid as solution A and a 70% acetonitrile solution containing 0.085% trifluoroacetic acid as solution B, with a flow rate of 1 ml/min and a linear gradient of solution B from 44% to 70% in 13 minutes. This procedure cleaved 85% of GP97ompPR and at the end of the reaction a peak corresponding to RHHGP[G] [SEQUENCE ID NO:25] was obtained (Figure 21 A).

Attachment to Reply and Amendment dated December 26, 2002

Marked-up Copy

Page 33, Paragraph Beginning at Line 14

By using RHHGP[G] [SEQUENCE ID NO:25] as an intermediate, GLP-1(7-37) having a purity of 99% was obtained at ease and at a high yield. Since HPLC is not used in the purification of the present invention, it is, needless to say, easy to scale up to an industrial scale.

Page 33, Paragraph Beginning at Line 26

RHHGP[G] [SEQUENCE ID NO:25] obtained in Example 4 was converted to RHHGP-1 [SEQUENCE ID NO:25] using an amidation enzyme. In order to determine the reaction condition for the case wherein RHHGP[G] [SEQUENCE ID NO:25] was used as a substrate, optimization was carried out for pH, temperature, the concentration of copper sulfate, catalase concentration, substrate concentration, L-ascorbic acid concentration, and the concentration of the amidation enzyme in a reaction volume of 0.5 ml. Separation of RHHGP[G] [SEQUENCE ID NO:25] and RHHGP-1 [SEQUENCE ID NO:25] and analysis thereof were carried out using an ion exchange HPLC column (Poros S/H, Perceptive Biosystems) in the presence of 30 mM Britton-Robinson buffer (referred to hereinafter as the BR buffer) excluding barbital at a pH gradient elution (6.0 to 9.0).

Attachment to Reply and Amendment dated December 26, 2002

Marked-up Claims 1, 18, 20, 21, 23, 25 and 26

1. (Amended) A process for producing a peptide having a desired biological activity, comprising the steps of:

(1) culturing cells transformed with an expression vector having [the] a nucleotide sequence encoding a peptide of interest that has a helper peptide added thereto, [or a fusion protein that has a protective peptide further added to the peptide of interest that has a helper peptide added thereto;] and then harvesting said peptide of interest that has a helper peptide added thereto [or said fusion protein] from said culture, wherein the helper peptide has 5 to 50 amino acid residues and is designed so that the isoelectric point of the peptide of interest that has helper peptide added thereto is between 8 and 12;

(2) [in the case wherein a fusion protein is obtained in step (1), cleaving off from said fusion protein the peptide of interest that has a helper peptide added thereto and the protective peptide, and purifying the peptide of interest that has a helper peptide added thereto as desired;

(3)] cleaving off from the peptide of interest that has [a] the helper peptide added thereto obtained in step (1) [or step (2)], the helper peptide and the peptide of interest [, and purifying the peptide of interest as desired]; and

[(4)] (3) purifying the peptide of interest obtained in step [(3)] (2).

18. (Twice Amended) The process according to claim [12] 1, wherein an ion exchange resin is used in the purification process.

Attachment to Reply and Amendment dated December 26, 2002

Marked-up Claims 1, 18, 20, 21, 23, 25 and 26

20. (Twice Amended) The process according to claim [12] 1, wherein a reverse phase chromatography or a hydrophobic chromatography is used in the purification process.

21. (Twice Amended) The process according to claim [12] 1, wherein a surfactant and/ora salt is added to maintain the solubility of the peptide of interest.

23. (Thrice Amended) The process according to claim 1, wherein endotoxin is present in the [final purified product] peptide of interest obtained in step (3), and wherein the content of endotoxin [in the final purified product] is not greater than 0.03 units/mg.

25. (Amended) An expression vector comprising a nucleotide sequence encoding a peptide of interest that has a helper peptide added thereto, [or a fusion protein that has a protective peptide further added to the peptide of interest that has a helper peptide added thereto] wherein the helper peptide has 5 to 50 amino acid residues and is designed so that the isoelectric point of the peptide of interest that has the helper peptide added thereto is between 8 and 12.

26. (Amended) A prokaryotic or a eukaryotic cell transformed with an expression vector comprising a nucleotide sequence encoding a peptide of interest that has a

Attachment to Reply and Amendment dated December 26, 2002

Marked-up Claims 1, 18, 20, 21, 23, 25 and 26

helper peptide added thereto, [or a fusion protein that has a protective peptide further added to the peptide of interest that has a helper peptide added thereto] wherein the helper peptide has 5 to 50 amino acid residues and is designed so that the isoelectric point of the peptide of interest that has the helper peptide added thereto is between 8 and 12.